

AMENDMENTS TO THE CLAIMS

1. (Currently amended) A method for amplifying a microRNA molecule to produce DNA molecules, the method comprising the steps of:

(a) producing a first DNA molecule that is complementary to a target microRNA molecule using primer extension with an extension primer comprising a first portion having a length from 3 to 17 nucleotides selected to hybridize to a portion of the target microRNA molecule and a second portion that hybridizes to the complement of a universal forward primer; and

(b) amplifying the first DNA molecule to produce amplified DNA molecules using the universal forward primer and a reverse primer, wherein the reverse primer is selected to specifically hybridize to a portion of the first DNA molecule that is complementary to the target microRNA molecule under defined hybridization conditions, wherein at least one of the universal forward primer and the reverse primer comprises at least one locked nucleic acid molecule.

2. (Canceled)

3. (Original) A method of Claim 1 wherein the primer extension uses an extension primer having a length in the range of from 10 to 100 nucleotides.

4. (Original) A method of Claim 1 wherein the primer extension uses an extension primer having a length in the range of from 20 to 35 nucleotides.

5. (Canceled)

6. (Previously presented) A method of Claim 1 wherein the first portion of the extension primer has a length in the range of from 6 to 17 nucleotides.

7. (Canceled)
8. (Previously presented) A method of Claim 1 wherein the second portion of the extension primer has a length of from 18 to 25 nucleotides.
9. (Previously presented) A method of Claim 1 wherein the second portion of the extension primer has a nucleic acid sequence comprising the nucleic acid sequence of SEQ ID NO:1.
10. (Original) A method of Claim 1 wherein the universal forward primer has a length in the range of from 16 nucleotides to 100 nucleotides.
11. (Original) A method of Claim 1 wherein the universal forward primer consists of the nucleic acid sequence set forth in SEQ ID NO:13.
12. (Previously presented) A method of Claim 1 wherein the universal forward primer hybridizes to the complement of the second portion of the extension primer.
13. (Currently amended) A method of Claim [[2]] 1 wherein the universal forward primer comprises at least one locked nucleic acid molecule.
14. (Currently amended) A method of Claim [[13]] 1 wherein the universal forward primer comprises from 1 to 25 locked nucleic acid molecules.
15. (Original) A method of Claim 1 wherein the reverse primer has a length in the range of from 10 nucleotides to 100 nucleotides.
16. (Currently amended) A method of Claim [[2]] 1 wherein the reverse primer comprises at least one locked nucleic acid molecule.

17. (Currently amended) A method of Claim [[16]] 1 wherein the reverse primer comprises from 1 to 25 locked nucleic acid molecules.

18. (Canceled)

19. (Original) A method of Claim 1 further comprising the step of measuring the amount of amplified DNA molecules.

20. (Original) A method of Claim 1 wherein amplification is achieved by multiple successive PCR reactions.

21. (Currently amended) A method for measuring the amount of a target microRNA in a sample from a living organism, the method comprising the step of measuring the amount of a target microRNA molecule in a multiplicity of different cell types within a living organism, wherein the amount of the target microRNA molecule is measured by a method comprising the steps of:

(1) producing a first DNA molecule complementary to the target microRNA molecule in the sample using primer extension with an extension primer comprising a first portion having a length from 3 to 17 nucleotides selected to hybridize to a portion of the target microRNA molecule and a second portion that hybridizes to the complement of a universal forward primer;

(2) amplifying the first DNA molecule to produce amplified DNA molecules using the universal forward and a reverse primer, wherein the reverse primer is selected to specifically hybridize to a portion of the first DNA molecule that is complementary to the target microRNA molecule under defined hybridization conditions, wherein at least one of the universal forward primer and the reverse primer comprises at least one locked nucleic acid molecule; and

(3) measuring the amount of the amplified DNA molecules.

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22. (Canceled)

23. (Original) The method of Claim 21, wherein the amount of the amplified DNA molecules are measured using fluorescence-based quantitative PCR.

24. (Original) The method of Claim 21, wherein the amount of the amplified DNA molecules are measured using SYBR green dye.

25–42. (Canceled)